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Original research paper

SUBCRITICAL WATER EXTRACTION OF DANDELION (*TARAXACUM OFFICINALE* L.) FLOWERS: INFLUENCE OF TEMPERATURE ON POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY

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Abstract: Dandelion (*Taraxacum officinale* L.) has a long history in traditional herbalism worldwide. Described as non-toxic, the herb has been consumed in various forms as a valuable source of nutrients, minerals and vitamins, the consumption of which may help prevent or reduce the risk of complex diseases such as cancer, obesity, arthritis, hepatitis, cardiovascular and gastrointestinal disorders.
All parts of the dandelion herb are edible and contain flavonoids, phenolic acids, alkaloids and terpenes, with the best-studied extracts being from leaves and roots. The most abundant phenolic acids are hydroxycinnamic acid derivatives, especially chicoric acid, chlorogenic acid and caffeic acid. Luteolin and its glucosides are more abundant in extracts from dandelion leaves and flowers.
This study aimed to investigate the influence of temperature of subcritical water extraction of dandelion flowers in the interval of 110-160 ° C. The parameters analysed were total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) and the DPPH radical scavenging activity, determined by UV-spectrophotometry. The highest TPC (36.37 mg GAE/g DW) and TAC (76.80 mg AAE/g DW) were noted in extracts obtained at 140 ° C, while the highest TFC of 10.95 mg RE/g DW was observed when extraction was performed at 130 ° C. As for DPPH scavenging activity, the highest value was for the extraction temperature of 110 ° C (0.906 mg AAE/g DW), and the lowest for the extract obtained at 160° C (0.718 mg AAE/g DW).
The results of this study suggest that dandelion flower extracts obtained at moderate temperatures (130-140 ° C) with subcritical water have the highest polyphenol content and antioxidant activity.

Key words: *dandelion flowers, subcritical water extraction, polyphenolic content, antioxidative activity*

INTRODUCTION

The common dandelion (*Taraxacum officinale* L.) is a perennial herbaceous plant from the Asteraceae family that is widespread throughout the world and is often regarded as a weed. It has long been consumed as a dietary medicinal and edible plant. Nowadays, dandelion is used to produce various supplements due to its health-promoting properties, including anti-inflammatory, anti-rheumatic, anti-oxidant, anti-carcinogenic, diuretic, choleretic, laxative

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and hypoglycemic activities (Li, Chen & Waterhouse, 2022).

Besides being used as a remedy, dandelion flowers, leaves and roots can also be processed into various foods. The leaves can be eaten fresh as a salad, the roots can be roasted and used as an additive in coffee preparation (Lis & Olas, 2019; Williams, Goldstone & Greenham, 1996), and the flowers can be used to make liqueurs or jams (Martinez et al., 2015), as a flavouring in frozen dairy desserts, sweets, cheese (Lis & Olas, 2019), as an additive in beer production or even fermented into wine (González-Castejón, Visioli & Rodriguez-Casado, 2012).

Various classes of bioactive compounds are present in the dandelion plant, namely polyphenols, flavonoids, terpenoids, vitamins, carotenoids, carbohydrates and minerals (Martinez et al., 2015). It has been reported that different parts of the herb (flower, leaf, stem and root tissue) contain different compounds at various concentrations (Williams et al., 1996). Dandelion roots are a rich source of sesquiterpene lactones, mainly eudesmanolides, guaianolides and esterified germacranolides, which are unique to the plant and are responsible for the bitterness of the herb (Lis & Olas, 2019; Sharifi-Rad et al., 2018). The roots also contain a high proportion of inulin. The highest concentrations of hydroxycinnamic acid derivatives, particularly chicoric acid, chlorogenic and caffeic acids, and sesquiterpene lactones, which generally occur as glycosides, have been found in the leaves and petals (Lis & Olas, 2019). The petals also contain natural rubber (Martinez et al., 2015). Dandelion leaf extracts are also rich in flavonoids (luteolin-7-*O*-glucoside, luteolin-7-diglucoside and luteolin) and terpenoids. Flower extracts with their high polyphenol content are also a rich source of hydroxycinnamic acid derivatives, luteolin and its glycosides, β-carotene and terpenoids (Lis & Olas, 2019).

Subcritical water extraction (SWE), a novel environmentally friendly technique, is gaining popularity due to its safety, low price and no negative impact on the environment. The polarity and dielectric constant of water under high temperature and pressure below the critical point (Tc = 374.15 °C, Tp= 22.1 MPa) change (Lachos-Perez et al., 2018). Temperature is the most important parameter in the extraction process that influences the efficiency of extraction. High temperatures can cause the degradation of some heat-sensitive compounds. The pressure of water in a subcritical state must be high enough to keep the water in its liquid state (Majeed et al., 2024).

Chen et al. (2023) proposed subcritical water extraction of polysaccharides from *Taraxacum mongolicum Hand.-Mazz.* leaves at 120 °C and reported the anti-cancer activity of the extracts obtained. To our best knowledge, there are no studies in the scientific literature on the extraction of bioactive compounds from dandelion flowers with subcritical water. This study aimed to prepare extracts from dandelion (*Taraxacum officinale* L.) flowers using subcritical water at different temperatures (110-160 °C) and to investigate the effect of temperature on the total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) and DPPH radical scavenging activity of the extracts obtained.

MATERIALS AND METHODS

Plant material

The plant material, common dandelion flowers, was collected in April 2020 on the mountain Fruška Gora, Banstol, Serbia (45°9'32"N, 19°57'53"E). The flowers were air-dried in the shade on a cheesecloth until their weight was constant. The dried flowers were ground with a laboratory blender and stored in a paper bag at room temperature until extraction.

Chemicals and reagents

Gallic acid and rutin trihydrate were purchased from Dr. Ehrenstorfer GmbH (Ausburg, Germany). Ascorbic acid and 2,2-diphenyl-1-picrylhydrazil reagent (DPPH) were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent was purchased from Lachner (Neratovice, Czech Republic). Sodium carbonate and aluminium chloride hexahydrate were purchased from Alpha Aesar GmbH & Co KG (Karlsruhe, Germany). Sulphuric acid was purchased from Sigma-Aldrich (Steinheim, Germany). Sodium phosphate dodecahydrate and ammonium molibdate tetrahydrate were purchased from Zorka (Šabac, Serbia). Nitrogen under pressure (99.999%) was supplied by Messer (Bad Soden, Germany). All other chemicals were of analytical reagent quality.

Subcritical water extraction (SWE)

The SWE of dandelion flowers was carried out in a customised high-pressure reactor, model FCF (ZZDK Instrumental Equipment, Zhengzhou City, Henan Province, China), a mechanically stirred reaction device operating at a maximum pressure of 220 bar, a maximum temperature of 450 °C, a heating power of 1500 W and a stirring speed of 0-1000 rpm.

The reaction vessel consists of a stainless steel vessel body with a maximum capacity of 1 litre, with a heating ring around it and a vessel lid. The plant material and distilled water were added to the reaction vessel in a ratio of 1:20 (*w/w*). The vessel was closed by turning a handwheel of the lifting device, which moved the vessel lid over the vessel, and the lid was secured with eight screws. After closing, the extraction cell was pressurised to a constant pressure of 10 bar with nitrogen via the gas inlet valve. The reactor is equipped with a manometer, a safety valve, and two water-cooling systems. One system is used to cool the reactor motor during extraction, especially at high temperatures and rotation speeds during long working periods. The other system is for cooling the process vessel after extraction. The heating speed and the final temperature (110-160 °C) were automatically controlled via a digital display controller. The accuracy of temperature control was ± 1 °C. The speed of the rod mixer (180 rpm) was also shown on the speed display of the controller. After the operating temperature was reached (110-160 ° C), the extraction time (20 min) was measured. After extraction, the reaction cell was cooled down to 20 ± 2 °C using the water-cooling system and the pressure was released by opening the pressure relief valve. The extracts obtained were filtered through a Whatman filter paper (class 1) and stored in polyethylene bottles in the refrigerator (4 °C) for further analyses.

Total phenolic content (TPC)

The total phenolic content of dandelion flower extracts from subcritical water was measured using the Folin-Ciocalteu method (Li et al., 2007). The reaction mixture was prepared by mixing 400 µl of the extract or standard solution with 2 ml of diluted Folin-Ciocalteu reagent (1:10, *v/v*) and adding 1.6 ml of sodium carbonate solution (7.5%, *w/w*) after 4 minutes. The blank sample was prepared with distilled water instead of the extracts. The mixtures were incubated at room temperature for 90 minutes for colour development and the absorbance was measured at 765 nm. Measurements were performed in triplicate for each sample. The TPC was calculated by interoplating the measured sample absorbance into a calibration curve ($A = 0.0115\gamma + 0.0134$, $r^2 = 0.9996$) defined using standard solutions of gallic acid for the concentration range 0 – 200 mg/litre. The results were expressed as mg gallic acid equivalent per gramme dandelion flower dry weight (mg GAE/g DW) and calculated as mean ± SD.

Total flavonoid content (TFC)

The total flavonoid content in the dandelion flower extracts was determined by a rapid colourimetric method using $AlCl_3$ (Benmerzoug, Švarc-Gajić, Nastić, Guettaf & Harzallah, 2020) by adding 2 ml of a 2% $AlCl_3$ solution to 2 ml of the extract or standard solution. The blank sample was prepared by mixing 2 ml of distilled water with $AlCl_3$. After 10 minutes, the absorbance was measured at 430 nm. Measurements were performed in triplicate for each sample. Rutin trihydrate (0 – 125 mg/l) dissolved in distilled water was used as a standard ($A = 0.0084\gamma - 0.0013$, $r^2 = 1$). The results were expressed as mg rutin equivalent per gramme dry weight of dandelion flowers (mg RE/g DW) and calculated as mean ± SD.

Total antioxidant capacity (TAC)

The total antioxidant capacity of dandelion flower extracts from subcritical water was determined using the phosphomolybdenum method (Prieto, Pineda & Aguilar, 1999). Aliquots of 0.3 ml aqueous extract or standard solution were mixed with 3 ml of the reagent solution consisting of 0.6 mol/l sulphuric acid, 28 mmol/l sodium phosphate solution and 4 mmol/l ammonium molybdate. The mixtures were incubated at 95 °C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm and compared with a blank sample prepared by replacing the sample with an appropriate volume of distilled water. All measurements were performed in triplicate. Ascorbic acid (10 – 100 mg/l) was used as a standard. The calibration curve obtained was as follows: $A = 0.004\gamma - 0.0221$ ($r^2 = 0.9982$). The results were expressed as mg ascorbic acid

equivalents per gramme dry weight of dandelion flowers (mg AA/g DW) and calculated as mean ± SD.

DPPH radical scavenging activity

For the DPPH radical scavenging activity of dandelion flower extracts, the method described by Moreira et al. (2018) was used with slight modifications. In the original method, trolox was used as a standard to determine the calibration curve, while ascorbic acid (10-50 mg/l) was used in this study. A volume of 250 µl of each sample/standard solution was mixed with 2000 µl of an ethanolic DPPH solution (0.04 mg/ml). The mixture was left in the dark for 30 minutes and the absorbance was measured at 517 nm. Each extract was analysed in triplicate (mean ± SD). The calibration curve obtained was as follows: $A = 0.9802 – 0.0179\gamma$ ($r^2 = 0.9976$). The results were expressed as mg ascorbic acid equivalents per gramme of dry dandelion flowers (mg AAE/g DW). The blank sample consisted of 96% ethanol.

RESULTS AND DISCUSSION

Five different dandelion flower extracts (DFE) were prepared at different temperatures with subcritical water: DFE110 (prepared at 110 °C), DFE120 (prepared at 120 °C), DFE130 (prepared at 130°C), DFE140 (prepared at 140 °C) and DFE160 (prepared at 160 °C). The extraction time was 20 minutes, the nitrogen pressure was 10 bar, the rotation frequency was 180 rpm and the ratio of sample to solvent was 1:20 (*w/w*) for all extractions.

The extracts prepared were analysed for their TPC, TFC, TAC and DPPH radical scavenging activity. Data were expressed as means ± standard deviations (SD) of three independent experiments for each analysis. One-way analysis of variance (ANOVA: single factor test) was used to compare means and determine significant differences ($p < 0.05$). The results of TPC and TFC of dandelion flower extracts are shown in Fig. 1 and 2, respecttively.

As shown in Fig. 1, there were significant differences in TPC between all samples, indicating the strong influence of temperature on the content of polyphenols in dandelion flower extracts obtained with subcritical water, as different polyphenols have different solubility and heat sensitivity. In addition, changing the temperature of the subcritical water also changes the polarity of the water, so that polyphenols of different polarities can be extracted. The TPC gradually increased from 24.69 mg GAE/g DW to 36.37 mg GAE/g DW with the increase in temperature from 110 °C to 140 °C. At 160 °C, a slight decrease in TPC was observed (35.07 mg GAE/g DW). As for TFC (Fig. 2), the value increased with increasing temperature and reached a peak value at 130 °C (10.95 mg RE/g DW), after which the TFC decreased slightly and reached the value of 9.52 mg RE/g DW for DFE160, which corresponds to the TFC of the extract obtained at

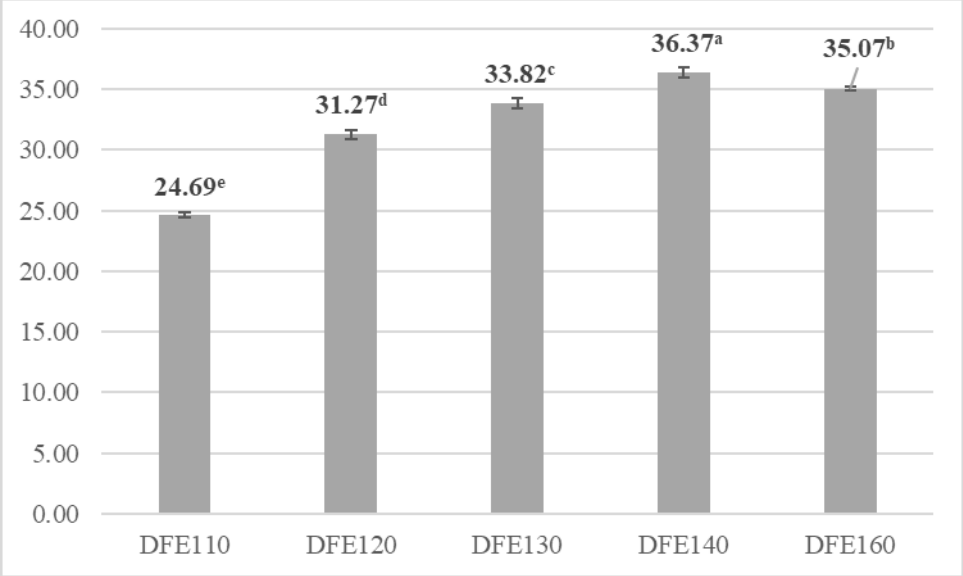

Figure 1. TPC of dandelion flower extracts obtained by SWE (mg GAE/g DW). The error bars indicate standard deviation (n = 3). Different letters (a, b, c, d, e) indicate a significant statistical difference in the observed data ($p < 0.05$)

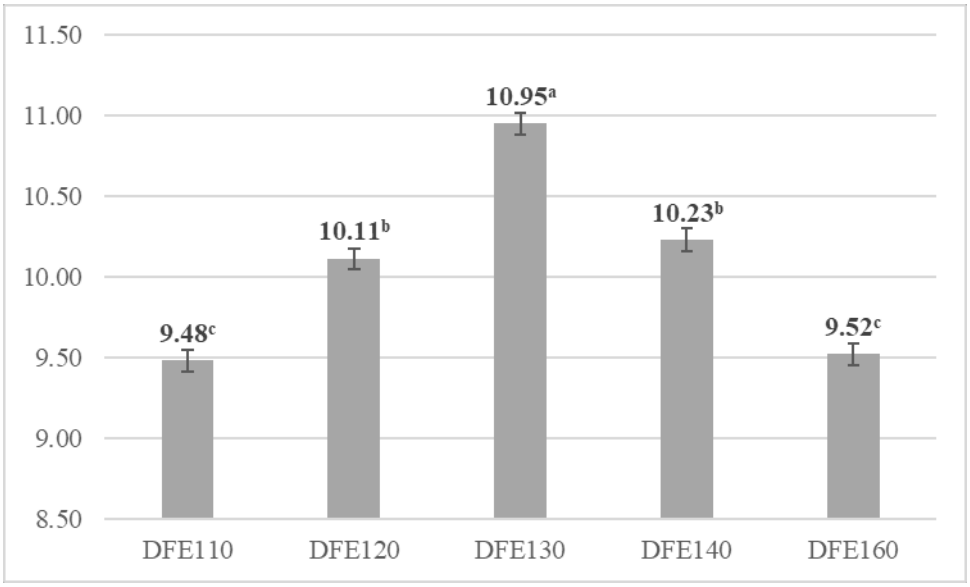

Figure 2. TFC of dandelion flower extracts obtained by SWE (mg RE/g DW). The error bars indicate standard deviation (n = 3). Different letters (a, b, c) indicate a significant statistical difference in the observed data ($p < 0.05$)

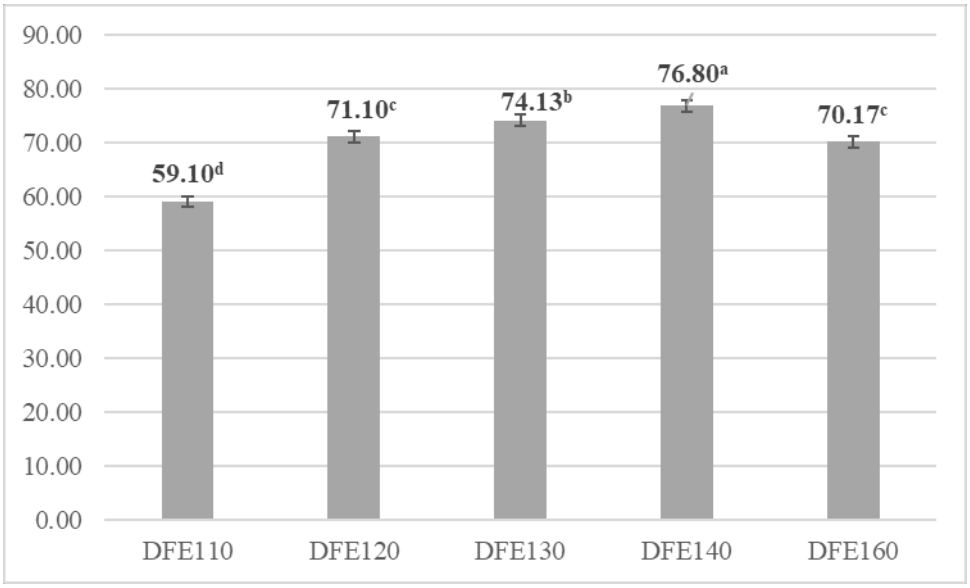

Figure 3. TAC of dandelion flower extracts (mg AAE/g DW). The values represent means (n = 3) ± SD. Different letters (a, b, c, d) indicate a significant statistical difference in the observed data ($p < 0.05$)

110°C. The total antioxidant capacity of the dandelion flower extracts obtained by subcritical water extraction is shown in Fig. 3, while the DPPH radical scavenging activity is shown in Fig. 4. As shown in Fig. 3, the TAC shows the same trend as the TPC, indicating that the polyphenols may be the main contributors to the antioxidant activity in the dandelion flower extracts. The highest TAC value was observed for the extract obtained at 140 ° C (76.80 mg AAE/g DW) and the lowest for the extract obtained at 110 °C (59.10 mg AAE/g DW). Regarding the DPPH radical scavenging activity (Fig. 4), the highest value was observed for DFE110 (0.906 mg/g DW), which was in contrast to the total antioxidant capacity. The lowest value was observed for the extract obtained at 160 °C (0.718 mg AAE/g DW).

At higher temperatures, thermostlabile polyphenols could be degraded and new compounds (Maillard reaction products) could be formed, which may have an antiradical effect (Jia, Guo, Zhang & Shi, 2023). Different extraction temperatures may also have influenced the type of polyphenols extracted.

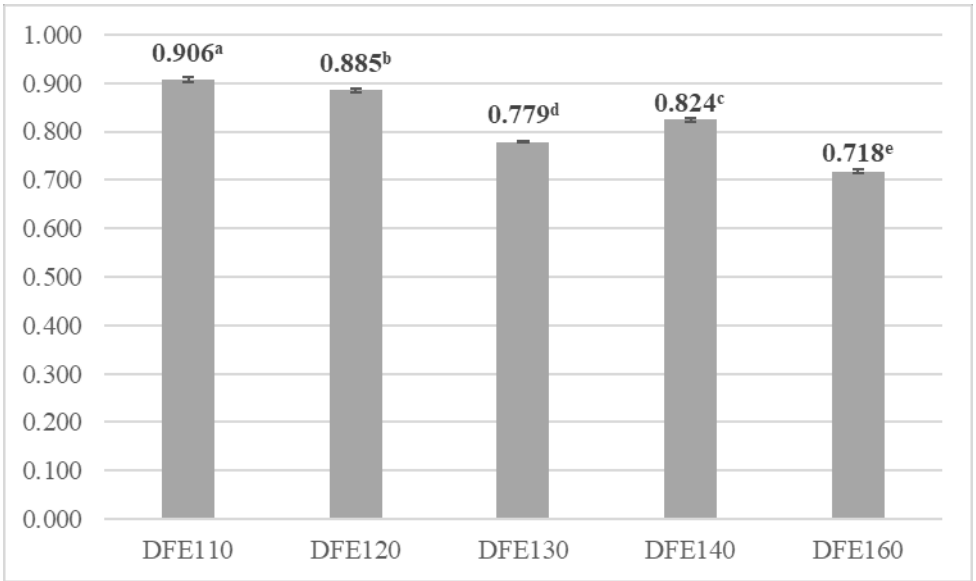

Figure 4. DPPH radical scavenging activity of dandelion flower extracts (mg AAE/g DW). The values represent means (n = 3) ± SD. Different letters (a, b, c, d) indicate a significant statistical difference in the observed data ($p < 0.05$)

In addition, the results of this study suggest the possible presence of thermolabile, non-phenolic free radical scavengers (carotenoids, vitamin C and vitamin E) in dandelion flower extracts extracted at lower temperatures (110° C and 120° C), which could react with the DPPH reagent but degrade at temperatures above 130° C.

Most of the studies available in the scientific literature tend to focus on the evaluation of the therapeutic effects and pharmacological profile of dandelion extracts (Bušić et al., 2018; Biel, Jaroszewska, Łysoń, & Telesiński, 2017). Few studies focus on the polyphenols in *Taraxacum officinale* L. flower extracts.

In the work of Dedić, Džaferović and Jukić (2022), who carried out a maceration extraction at room temperature with aqueous ethanol solution 50% (*v*/*v*) as a solvent for 4 hours, the TPC of the flower extract was 26.08 mg GAE/g. This value is comparable to the 24.69 mg GAE/g obtained for the lowest temperature of the SWE used in our study. The value of TFC (4.98 mg quercetin equivalent/g) reported by the same authors was not comparable with our results because different units were used to express TFC.

Zhu et al. (2024) performed ultrasonic extraction of unfermented and fermented dandelion at 20 kHz for 30 minutes in distilled water with a solid-liquid ratio of 1:100 (*w*/*v*). The TPC of unfermented dandelion was 12.47 mg GAE/g, while the TFC was 19.47 mg RE/g. Compared to these values, the TPC obtained in our study was higher (36.37 mg GAE/g), indicating the great efficiency of SWE in the extraction of polyphenols. On the other hand, the TFC value obtained in our study was significantly lower compared to the values obtained by Zhu et al. (10.95 mg RE/g compared to 19.47 mg RE/g), which could be due to the high thermolability of flavonoids.

The study by Miłek, Marcinčáková and Legáth (2019) confirmed the significant antioxidant potential of extracts from dandelion flowers and leaves, although the results could not be compared with those of our study due to the different units used. These authors determined a TPC value of 0.229 mg CE/g (catechin equivalent/g) for extracts obtained with Triton X-100 as an aqueous extraction modifier (2%) and a value of 0.385 mg CE/g for extracts obtained with acetone (30%). The antiradical DPPH activity of the same extracts was measured in mg Trolox equivalents per g: 0.294 mg TE/g was determined for the triton X-100 dandelion flower extract and 0.892 mg TE/g for the acetone extract.

Hu and Kitts (2005) determined the TPC value of 195.4 mg GAE/g of the ethanolic extract of dandelion flowers confirming the results of Williams et al. (1996), namely the presence of luteolin, luteolin-7-glucoside, caffeic acid and chlorogenic acid by HPLC analysis.

In addition to the quantitative differences in TPC, TFC, TAC and DPPH radical scavenging activity between the dandelion flower extracts obtained in this study, they almost certainly differ qualitatively, which could be investigated by analysing the polyphenolic profile using chromatography methods.

CONCLUSIONS

In the present study, dandelion flowers were extracted with subcritical water at different temperatures (110-160 °C). The effect of applied temperature on total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) and DPPH radical scavenging activity was significant. The highest TPC (36.37 mg GAE/g DW) and TAC (76.80 mg AAE/g DW) were observed in extracts obtained at 140 ° C, while the highest TFC of 10.95 mg RE/g DW was observed when extracted at 130 °C, suggesting that the optimum temperature for SWE of dandelion flowers is between 130 °C and 140 °C. The highest value of DPPH radical scavenging activity was observed at an extraction temperature of 110 °C (0.906 mg AAE/g DW) and the lowest value in the extract obtained at 160 °C (0.718 mg AAE/g DW). All dandelion flower extracts from this study can be consi-dered as rich sources of polyphenols and flavornoids with high antioxidant activity, especially those obtained at moderate temperatures (130-140 °C), suggesting that subcritical water is a powerful and safe extraction technique. As abundant sources of bioactive compounds, subcritical water extracts from dandelion flowers could be considered potential additives in functional foods, pending confirmation of their safety through standardized tests.

AUTHOR CONTRIBUTIONS

Conceptualization, T.Ž.B-B.; Methodology, T.Ž.B-B. and J.V.Š-G.; Investigation, formal analysis, validation, writing-original draft preparation, T.Ž.B-B.; Writing-review and editing, J.V.Š-G.; Supervision, J.V.Š-G.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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EKSTRAKCIJA CVETA MASLAČKA (*TARAXACUM OFFICINALE* L.) SUBKRITIČNOM VODOM: UTICAJ TEMPERATURE NA SADRŽAJ POLIFENOLA I ANTIOKSIDATIVNU AKTIVNOST

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Sažetak: Maslačak (*Taraxacum officinale* L.) ima dugu istoriju primene u fitoterapiji širom sveta. Ova biljka, koja se smatra netoksičnom, upotrebljava se u različitim oblicima kao značajan izvor nutrijenata, minerala i vitamina, a redovnom konzumacijom pomaže u prevenciji i smanjenju rizika složenih oboljenja kao što su kancer, gojaznost, artritis, hepatitis, kardiovaskularne i bolesti gastrointestinalnog sistema.

Svi delovi biljke maslačak su jestivi i sadrže flavonoide, fenolne kiseline, alkaloide i terpene, od čega su ekstrakti lista i korena najčešće proučavani. Najzastupljenije fenolne kiseline čine derivati hidroksicinamične kiseline, posebno cikorične, hlorogenske i kafeinske kiseline. Luteolin i glikozidi luteolina su zastupljeniji u ekstraktima lista i cveta maslačka.

Cilj ovog rada bio je da istraži uticaj temperature subkritične vode prilikom ekstrakcije cvetova maslačka u intervalu 110-160 ° C. Sadržaj ukupnih fenola (TPC), sadržaj ukupnih flavonoida (TFC), ukupna antioksidativna aktivnost (TAC) i sposobnost hvatanja DPPH radikala su određeni spektrofotometrijski.

Najvišu vrednost TPC (36,37 mg GAE/g DW) i TAC (76,80 mg AAE/g DW) je pokazao ekstrakt dobijen na temperaturi 140 °C, dok je najviša vrednost dobijena TFC uočena za ekstrakt dobijen na 130 ° C (10,95 mg RE/g DW). Najveću sposobnost hvatanja DPPH radikala je pokazao ekstrakt dobijen na temperaturi 110 ° C (0,906 mg AAE/g DW), dok je najmanju sposobnost pokazao ekstrakt dobijen na 160 °C (0,718 mg AAE/g DW). Rezultati ove studije pokazuju da ekstrakti cveta maslačka dobijeni na srednjim temperaturama (130-140 °C) subkritičnom vodom imaju najviši sadržaj polifenola i najvišu antioksidativnu aktivnost.

Ključne reči: *cvet maslačka, ekstrakcija subkritičnom vodom, sadržaj polifenola, antioksidativna aktivnost*

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